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PYRIDOSTIGMINE AND WARM WATER DIVING PROTOCOL 90-05: IV. PHYSICAL PERFORMANCE

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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The effect of pretreatment with pyridostigmine on exercise performance was studied on 10 heat-acclimated divers performing light to moderate work for 3 h in 34.4 °C (94 °F) water at a depth of 20 feet of seawater (fsw) (1.6 ATA). Dives began after a 4-hour surface exposure in 37.8 °C (100 °F) air. Handgrip strength was measured during the predive period. Each subject was tested twice, in a balanced order among subjects. One trial was conducted after pretreatment with pyridostigmine bromide (PYR) for 2 days (30 mg, every 8 h). The second trial was done after receiving a placebo (PLA) over a similar time course. An interval of 5 days elapsed between trials. During both trials subjects breathed 100% oxygen (PO_2 = 1.6 ATA) at depth. A third trial was subsequently conducted, in the absence of drug and while breathing air at 20 fsw, to determine whether air or 100% O_2 provided a cardiorespiratory advantage during warm water exercise. Subjects sat in a semi-recumbent position throughout the dives. Leg exercise was conducted using an electronically braked cycle ergometer.

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During the first 2 h of immersion, subjects exercised in a repeated pattern of 30 min at a workload of 25 watts (W) followed by 10 min of rest. This workload approximated fin swimming at 0.6 knots. During the last hour the work pattern was 5 min at 25 W, 10 min at 1.0 W/kg, and 5 min rest. The higher workload approximated fin swimming at 1.0-1.1 knots. Heart rate was measured every 5 min during immersion. Minute ventilation (V_E), respiratory timing, and oxygen consumption (V_{O_2}) were measured during each work and each rest period. Relative perceived exertion was unaffected by either drug or breathing gas treatments. These findings indicate that pretreatment with pyridostigmine does not limit the capability to perform light-moderate exercise in warm water. Furthermore, breathing air instead of 100% O_2 does not appreciably modify the cardiorespiratory responses to this exercise paradigm.

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1. INTRODUCTION

Pyridostigmine bromide is a drug used clinically to treat myasthenia gravis. It is also authorized for issue to military personnel as a prophylactic measure against organophosphate nerve agents, although at a dose lower than used clinically.[14] The principal action of the drug is to inhibit cholinesterase activity,[21] which could affect any physiological mechanism associated with cholinergic neural or muscular processes.

Studies on healthy humans pretreated with pyridostigmine (30 mg every 8 h) have shown that resting skin blood flow and skin temperature were lower with the drug than with placebo tests during exposure to warm air environments.[18,23] Sweating rate has been shown to increase after drug treatment.[8,11,23] Walking on a treadmill during heat exposure did not alter the rise in core temperature with the drug when compared to placebo conditions.[11] Running at 58-60% of $\dot{V}_{O_{2max}}$ in the heat did, on the other hand, result in a rectal temperature that was 0.1 °C higher after pyridostigmine ingestion than after ingesting a placebo.[18] Higher workloads have not been tested in humans to determine if endurance is altered by the drug. However, rats run to exhaustion after receiving pyridostigmine exhibited decreases in endurance time and more muscle damage than in untreated rats.[13] Thus far, no studies have been reported examining the effects of pyridostigmine during immersion.

There are two principal effects of immersion that influence the ability to both thermoregulate and perform exercise. First, translocation of peripheral blood into the thorax results in immersion diuresis. For immersions lasting longer than 2 h this diuresis can result in significant loss of body fluid.[9,10] Consequently, any associated decrease in

plasma volume might impair peripheral thermoregulation through circulatory alterations, or compromise the ability to maintain cardiac output during exercise.[8] Second, as water temperature approaches that of the skin the gradient for heat loss from the body is reduced. Increases in metabolic heat production during exercise must be met by corresponding increases in the rate of heat loss to prevent an increase in core temperature. In warm water (low skin-to-water temperature gradient) any increase in metabolic rate may result in a rise in core temperature because of the reduced capacity to dissipate heat. In addition, evaporative heat loss from sweating will not occur during immersion. It is well documented that rises in core temperature will result in decrements in physical performance.[3,11,13,18]

In hyperbaric environments the composition of the breathing gas can affect performance. Air or N_2O_2 mixtures at depths greater than 100 feet seawater (fsw) have been shown to limit physical performance due to inert gas narcosis and to increased gas density.[12,22,25,31] Increases in the inspired partial pressure of oxygen can increase endurance and lessen the rate of muscular fatigue both at the surface and at depth.[1,30] Breathing 100% O_2 is limited to depths shallower than 60 fsw because of the risk of acute oxygen toxicity, which increases as depth or exposure time increase.

Interestingly, some of the reported side effects of pyridostigmine are the same as for acute oxygen toxicity (e.g. nausea, tremor, and visual disturbances).[2,17,27]

It was reasoned that any undesirable effects of pyridostigmine, thermal stress, or hyperbaric oxygen exposure, when considered alone, might be insufficient to limit work tolerance. However, any additive effects between these parameters might produce

measurable changes during exercise that would limit a diver's capacity to perform underwater work.

The purpose of this study was to determine whether pretreatment with pyridostigmine altered the cardiorespiratory responses to light-moderate leg exercise done in 34.4 ° C (94 ° C) water at a depth of 20 fsw while breathing 100% oxygen. Immersions were designed to last 3 h in order to reasonably test endurance. The light-moderate workloads approximated fin swimming at 0.6-1.1 knots (19-34 m/min).[19] A water temperature of 34.4 ° C represented a reasonable upper limit to be encountered in ocean diving. It also represents a temperature that, coupled with the proposed exercise profile, would not likely result in the rapid development of hyperthermia; thereby permitting enough time to evaluate any drug effects on performance. A depth of 20 fsw was chosen because breathing 100% O_2 for 3 h at this depth should have a low incidence of oxygen toxicity. Thus, a higher than expected incidence of toxicity might suggest an additive effect of drug and hyperoxia. Deeper depths increase the incidence of O_2 toxicity and, given the high individual variability to O_2 tolerance, would make separation of drug vs O_2 effects quite difficult.

A separate test was also done with the subjects breathing air at depth (no drug) to establish differences between breathing air vs 100% O_2 in warm water. Data obtained from the air dives also enabled a refined estimate of drug vs thermal effects by a cross-correlation analysis with air vs O_2 effects.

This report focuses on the exercise aspects of this study. An overview of the entire research protocol can be found in a separate report.[6] Details of thermal balance, cognitive performance, and hydration status are contained in separate reports.[6,24,29]

2. METHODS

a. Subjects: Ten U.S. Navy divers volunteered to participate in the study after being informed of objectives and procedures. The study was approved by the NMRI Committee for Protection of Human Subjects. Their physical characteristics are presented in Table 1. An additional two divers volunteered to participate in the air vs O_2 exposures, in the absence of drug ingestion. The study was conducted during September-October 1990.

b. Pre-exposure Conditions: Subjects were acclimated to the heat for 5 consecutive days prior to the first exposure, and on alternate days between exposures. Each daily acclimation consisted of approximately 90 min in 37.8 °C air at the surface while exercising on a cycle ergometer at a workload of 1.0-1.5 W/kg for 3 bouts of 25 min work, 5 min rest.

For two days prior to each exposure, subjects ate standardized meals and ate breakfast 2 h before the start of the exposure. All meals consisted of Meal-Ready-to-Eat (MRE) rations. Each meal averaged 1298 kcal; with 50, 35, and 15% of the calories derived from carbohydrate, fat, and protein, respectively. No alcohol or nicotine products were used during these 2-day periods. Caffeine was restricted to no more than 3 cups of coffee per day, with no caffeine consumed on the day of the exposure.

c. Exposure Profile: Two subjects were tested simultaneously during each 7-hour exposure. A 4-hour predive period at the surface breathing air (temperature = 37.8 ± 0.5 °C, relative humidity = 50%) preceded the immersed phase. The 3-hour immersed phase was conducted in the wet pot of the Man-Rated Chamber Complex. Water temperature was 34.4 ± 0.1 °C. Subjects sat in a semi-recumbent position with the top of their heads approximately 2-4 inches below the water surface. Subjects wore a full face mask and breathed gas through a demand regulator. The chamber was compressed to 1.6 ATA (20 fsw) with air.

Subjects wore camouflage utilities and footwear throughout the 7-hour exposures. A fluid intake regimen of 1 ℓ of water per hour was enforced during the 4-hour predive period. No fluid was ingested during the immersed phase, nor was any food eaten during the entire 7-hour exposure.

An interval of 5 days elapsed between the first and second exposures (drug & placebo trials), and an interval of 8 days occurred between the second and third exposures.

d. Immersed Exercise: After reaching a depth of 20 fsw, the subjects exercised intermittently on a calibrated electronically braked cycle ergometer (Pedalmate, Warren Collins) modified for underwater use.[28] The pedaling rate was 50 rpm during all tests. Three bouts of work/rest were conducted during the first 2 h of immersion, each consisting of 30 min exercise at a bike workload setting of 25 W, followed by 10 min rest. Previous experience had shown that this work rate would produce an exercise $\dot{V}_{O_2}$ of about 1.0 ℓ/min in the water.[7,20]

During the 3rd hour of immersion subjects performed 3 bouts of work/rest; each consisting of 5 min exercise at 25 W, 10 min at a workload of 1 W/kg, and 5 min rest. We have shown previously that a workload of 1 W/kg will produce a $\dot{V}_{O_2}$ of approximately 2.0 ℓ/min.[15] Following the last rest period the chamber was decompressed to the surface and the divers exited the water.

e. Test Trials: Three tests trials were conducted. The first two trials for the 10 subjects consisted of examining the effects of pyridostigmine vs placebo. Pretreatment with pyridostigmine involved the subjects ingesting a 30-mg tablet every 8 h for two days prior to exposure, with a final dose given at 0600 on the day of the test. An identical time pattern of dosing was followed for placebo trials. Placebo tablets were saccharin, which were similar in color and size to pyridostigmine tablets. During the dive phase of these trials 100% O_2 was breathed. Trials were done in a balanced order, with 5 subjects undergoing their first trial after ingesting the drug and the other 5 undergoing their first trial after ingesting a placebo. Subjects and all but one investigator were blind with respect to drug/placebo ordering. Ten consecutive days were utilized to conduct the first two trials. An interval of 5 days separated exposures for any subject.

The third trial was done in the absence of drug, while breathing air at depth. Six of the 10 subjects, plus the 2 additional divers, were studied during this trial. Eight days elapsed between the 2nd and 3rd trials for each subject. Otherwise, the experimental conditions were the same as for the first 2 exposures.

f. Measurements: Subjects reported to the laboratory about 2 h prior to the start of an exposure. After obtaining body weight, subjects drank 5 ml water/kg body weight.

Three ECG electrodes were placed on the sternum, and connections waterproofed in order to measure heart rate (HR). Heart rate was recorded every 5 min from the HR display of a cardiac monitor (Sensor Medics, Anaheim, CA).

A calibrated thermistor was inserted 15 cm beyond the anal sphincter to measure rectal temperature. Rectal temperature was recorded and displayed every minute by an automated digital computer system.[16]

Handgrip strength of the right hand was measured during the predive dry phase exposure. The handgrip dynamometer was calibrated before each exposure by suspending known weights from the handle and recording the tension reading on the dynamometer dial. Maximum grip strength was determined at the start of the exposure as the average value of three maximum contractions, each separated by about 1 min. A value equal to 80% of maximum strength was used to assess the decline in grip tension during a 1-minute sustained isometric contraction. Readings were obtained every 10 sec during these tests, which occurred at the start and at the 2nd and 4th hour of the predive exposure. No grip strength tests were conducted during the immersed phase.

Minute ventilation ($\dot{V}_E$) was obtained by directing the subjects' expired gas, via respiratory tubing, through a pneumotachograph (Model 3813, Hans Rudolph, Kansas City, MO). Free flow of gas through the demand regulator was eliminated by a check valve on the exhaust side of the regulator. The pneumotachograph was heated to 40 °C, and coupled to a 2-cm H_2O differential pressure transducer (Model DP45-18, Validyne). The output signal of the pressure transducer, proportional to flow, was converted from analog to digital and recorded on a computer at a sampling frequency of 30 Hz. The

pneumotachographs were calibrated at 20 fsw using a 3-liter respiratory syringe. Integration of the flow signal with respect to time for each breath provided tidal volume.[26] Sampling periods were 2 min each, and the summation of tidal volumes divided by 2 was used to derive $\dot{V}_E$. In addition, timing of the inspiratory and expiratory phases of each breath were determined. The 2-minute sampling periods occurred every 10 min during the first 2 h of exercise, and at the end of each workload and rest period during the last hour.

Oxygen consumption ($\dot{V}_{O_2}$) was determined during the 2-minute respiratory sampling periods by collecting the exhaled gas into a weather balloon located downstream of the pneumotachograph. Expired oxygen concentration was measured by a paramagnetic analyzer (Model 540A, Taylor Inst., Rochester, NY) calibrated with primary gravimetric standards.

Relative perceived exertion (RPE) was determined immediately after the end of each exercise bout. The 0-10 Borg scale[5] was used to quantify RPE, with 0 representing "very easy" and 10 representing "very difficult."

g. Abort Criteria: It was planned to abort any exposure if HR reached 90% of the subject's maximum value (registered during work-up $\dot{V}_{O_{2max}}$ tests). Exposures would be terminated prematurely if rectal temperature reached 39.5 °C for a period of 1 min.

h. Statistical Analyses: The effect of drug vs placebo on maximal grip strength was assessed by a paired t-test. A 2-way analysis of variance (ANOVA) was used to determine time and drug vs placebo effects on the decline in force during the isometric contractions.

Cardiorespiratory variables measured during immersion were segregated according to the 30-minute exercise periods at 25 W, to the 10-minute periods at 1 W/kg, and to the respective rest periods after each work period. Missing respiratory data points during the tests precluded an extensive analysis for a time effect within each period. Therefore, data for all subjects in each experimental group were averaged for the three 30-minute periods at 25 W, for the rest periods following 25 W exercise, for the three 10-minute periods at 1 W/kg, and for rest following 1 W/kg periods. One-way ANOVAs were performed on each of these averaged data sets to compare drug vs placebo and air vs O_2 effects. A similar statistical approach was used for HR data.

Statistical significance limits were set at p values less than 0.05. Data are reported as the mean ± SEM. Since there were a variable number of data points at each measurement, corresponding n values are reported in the text, table or figure legends.

3. RESULTS

Two subjects failed to complete the immersion phase because of malfunctions in the demand regulator (1 subject placebo/O_2, 1 subject no drug/air). Otherwise, all subjects completed the assigned in-water exercise. No terminations occurred because of high core temperature or high HR.

a. Grip Strength: (Dry phase only): Pyridostigmine did not significantly alter maximal grip strength in the dry (drug: 54.9 ± 2.5 vs placebo: 56.5 ± 3.0 kg). The magnitude of the decline in grip strength during the sustained isometric contraction, beginning at 80% of maximum, did not vary as a function of time for either drug or

placebo trials. There were no significant differences in the magnitude of the decline in tension between drug and placebo (4th hour in dry, drug: 14.0 ± 1.6 vs placebo: 12.0 ± 2.6 kg).

b. Minute Ventilation ($\dot{V}_E$): Figure 1 presents the mean data obtained during each measurement period for pyridostigmine, placebo, and air trials. Overall, there was little change in $\dot{V}_E$ over each of the 3 respective work cycles. There were no apparent differences between drug and placebo values.

Figure 2 (top panel) illustrates the $\dot{V}_E$ data averaged for each measurement period upon which statistical tests were made (n = 9 subjects). The light workload resulted in a $\dot{V}_E$ of 36-37 ℓ/min, while the moderate workload raised $\dot{V}_E$ to 51 ℓ/min. No significant differences in ventilation were noted between drug and placebo at any of the measurement periods.

Figure 1 indicates an apparent lower $\dot{V}_E$ during the first 2 h of immersion when breathing air. The averaged data shown in Figure 3 (top panel) revealed no significant differences between air and O_2, except for the rest periods after 25 W work cycles (REST A in figure). No other statistically meaningful differences were observed between air and O_2 trials.

c. Oxygen Consumption: ($\dot{V}_{O_2}$): Figure 4 summarizes the $\dot{V}_{O_2}$ data for each measurement period during immersion for the 3 test trials. No appreciable change with time occurred at each of the respective exercise periods. There were no apparent differences between pyridostigmine vs placebo trials. Figure 2 (lower panel) indicates

that, statistically, there were no differences between pyridostigmine and placebo trials for any exercise or rest period.

Figure 4 illustrates that, except for the first 30 min at 25 W, the only effect of breathing air instead of 100% O_2 was to produce a slightly lower $\dot{V}_{O_2}$ during the rest periods. Statistical analysis of the data shown in Figure 3 (lower panel) indicated that only significant effect occurred during the post 25 W rest period (REST A in figure). No meaningful differences were encountered at either workload.

d. Ventilatory Equivalent ($\dot{V}_E/\dot{V}_{O_2}$): The ratio of $\dot{V}_E$ to $\dot{V}_{O_2}$ was calculated for each of the available respiratory measurements. Figure 5 illustrates the comparison between pyridostigmine and placebo. As expected, the $\dot{V}_E/\dot{V}_{O_2}$ was appreciably higher at 25 W workloads than at 1 W/kg for both treatment conditions. Except for the 2nd work period at 25 W, there were no differences in $\dot{V}_E/\dot{V}_{O_2}$ between drug and placebo.

Figure 6 presents $\dot{V}_E/\dot{V}_{O_2}$ comparisons between breathing air and O_2 (placebo trials). Again there were no appreciable differences between air and O_2, except during the 2nd work period at 25 W.

e. Respiratory Patterns: Figure 7 presents tidal volume and breathing frequency for drug and placebo trials during immersion. Tidal volume averaged around 1.8-1.9 ℓ, with no difference between drug conditions. Breathing frequency was significantly higher at 1 W/kg than at 25 W, but otherwise there were no differences as a function of drug vs placebo. Qualitatively similar results were observed in the air vs O_2 trials (Figure 8).

The times for inspiration, expiration, and total respiratory cycle are shown in Figures 9 and 10. Rest periods were associated with slightly longer timing components, but no significant differences occurred with respect to drug vs placebo, nor air vs O_2 breathing.

Pyridostigmine did not produce significant changes in neural control of respiration, as reflected by mean inspiratory flow (V_T/V_i) and duty cycle time (T_i/T_{tot}, Figure 11). Likewise, there were no differences between air vs O_2 for these variables (Figure 12).

f. Heart Rate (HR): Figure 13 illustrates the average HR obtained during each measurement period for drug, placebo, and air trials. The only significant difference among the three trials was a higher HR at 1 W/kg when breathing air.

Table 2 provides the averaged HR data during each rest and exercise period. No significant differences were demonstrated between pyridostigmine and placebo for rest or exercise. As suggested by Figure 13, Table 2 indicates that the only statistically significant difference between air and O_2 trials occurred at 1 W/kg, with HR an average of 11 ± 4 beats/min higher when breathing air.

g. Relative Perceived Exertion (RPE): Figure 14 presents the relative perceived exertion (RPE) scores for subjects completing the two drug and one air trials in 34.4 °C water. For each of the three conditions there were no significant changes in RPE over time during the first 3 work periods at 25 W and during the last 3 periods at 1 W/kg. There was, as might be expected, a small but significant increase in RPE between the 3rd work period at 25 W and the first period at 1 W/kg. Pyridostigmine did not significantly affect RPE when compared to placebo trials. Likewise, there was no

significant difference between placebo (O_2) and air trials. While not shown in the figure, analysis was done on test sequence (irrespective of drug or gas) to determine whether repeated exposure to warm-water-altered RPE. No significant effect occurred going from the 1st to the 3rd trial for any subject.

4. DISCUSSION

The results of this study demonstrated that pretreatment with pyridostigmine did not significantly affect the divers' ability to perform light-moderate work in 34.4 °C water. One of the known side effects of this drug when given to normal individuals is muscle weakness and tremors.[2,17,27] Grip strength in the dry was not notably different between drug and placebo, indicating that strength was unaltered. This finding is consistent with a previous study conducted in the dry.[4]

Had pyridostigmine produced a noticeable decrease in leg muscle strength, one might have expected to see a decrease in endurance during the 3 h of immersion. Except for two cases of equipment malfunction, all subjects completed all exercise periods. The absence of any drug-related change in RPE suggests that the divers did not subjectively notice any increase in perceived effort, as might occur had muscle weakness been present. Likewise, the absence of significant changes in HR, $\dot{V}_E$, or $\dot{V}_{O_2}$ after pretreatment with the drug indicate that these physiological responses to exercise were unaltered.

Since pyridostigmine did not affect $\dot{V}_E$ or $\dot{V}_{O_2}$, one may conclude that the drug will not limit the ability to perform light to moderate work in warm water. Further, there

was no difference in $\dot{V}_E/\dot{V}_{O_2}$ between drug and placebo, suggesting that the ventilatory cost of performing exercise was unchanged. Based on the average values of $\dot{V}_{O_2}$ during each rest and exercise period, it was estimated that approximately 6.5 ft^3 of O_2 were consumed during this 3-hour dive profile. Thus, current oxygen sources in closed-circuit underwater breathing apparatuses (e.g. LAR-V contains about 10 ft^3 of O_2) would likely be sufficient to complete this type of dive. On the other hand, respiratory minute volume calculations predict that about 212 ft^3 of gas would be required for the dive paradigm if open-circuit scuba were used. This exceeds the normal capacity of a standard set of double scuba tanks (i.e. 160 ft^3 of air).

The lack of an effect of either drug or breathing gas on oxygen consumption indicates that the rates of metabolic heat production were similar among the test conditions. Therefore, it is not surprising that core temperatures were similar among conditions.[16]

Indirect evidence, offered through measurements of the timing components of the respiratory cycle, suggests that neural control of ventilation was unchanged by pretreatment with pyridostigmine or by breathing air at 20 fsw instead of 100% O_2. Given that the drug would not penetrate an intact blood-brain barrier,[27] this is putative confirmation that neither the high oxygen levels nor the thermal stress resulted in drug penetration into the central nervous system. The lack of any change induced by breathing air at 20 fsw is not surprising since inert gas narcosis would be virtually absent at this depth.

Changes in exercise HR were unimportant after pretreatment with pyridostigmine when compared to placebo trials. While the rate was slightly lower during the dry phase after drug treatment,[6,16] the effects of immersion and exercise were sufficient to override any drug effect, if present.

A number of studies have reported that HR is lower during rest and light-moderate exercise when breathing hyperoxic gas mixtures.[1,12,25] This effect of hyperoxia, in the present study, was demonstrable only at the higher workload.

It has been shown that immersion diuresis can result in a significant reduction in plasma volume in cold water, necessitating a higher HR to support exercise.[8] Immersions in 35 ° C water, however, result in only minor changes in plasma volume, although urine production is similar to that in colder water.[9,10] Plasma volume was reduced from pre- to post-exposure in the present study by about 2-3%,[24] which agrees with the previous warm water data. Such a minor change in plasma volume would likely have a negligible effect on maintenance of cardiac output (HR * stroke volume) during the 3-hour immersions. This conclusion is supported by noting that there was little change in HR during the 3 consecutive exercise periods at 25 W and during the 3 periods at 1 W/kg. If a notable reduction in plasma volume had occurred, then one would expect HR to gradually increase for a given workload since stroke volume would be decreased by an amount proportional to the reduction in plasma volume.

5. SUMMARY

a. Pretreatment with low dose pyridostigmine did not limit the ability of diver's to perform light to moderate leg exercise in 34.4 ° C water for 3 h at a depth of 20 fsw.

b. The drug did not significantly affect minute ventilation, oxygen consumption, tidal volume, heart rate, or perceived exertion.

c. Breathing air at 20 fsw did not significantly alter respiratory variables when compared to breathing 100% oxygen. Heart rate was 11 beats/min higher at the moderate workload (1 W/kg) when breathing air.

6. LAY LANGUAGE SUMMARY

Pyridostigmine is a drug that can be used as pretreatment prophylaxis against possible exposure to chemical nerve warfare agents. This study was conducted to determine if pretreatment with the drug would have adverse effects on a diver's ability to perform work in warm water.

Ten U.S. Navy divers participated in two dive tests, once after ingesting pyridostigmine (30 mg every 8 h) for two days, and once after taking a placebo (no drug). Each dive entailed a 4-hour exposure in 100 ° F air at the surface, followed by a 3-hour dive in 94 ° F water at a depth of 20 feet seawater. Divers breathed 100% oxygen at depth. Handgrip strength was measured during the predive period. Intermittent leg exercise was conducted at light-moderate work rates during the dive that simulated swimming at 0.6-1.1 knots (19-34 meters per min). Heart rate, respiratory minute ventilation, and oxygen consumption were measured during the exercise periods.

Handgrip strength was unaffected by pretreatment with the drug. Ingesting the drug did not alter measurements of heart rate or respiratory variables at either work rate. At the light work rate, heart rate averaged 112-115 beats per min, ventilation 36-38 ℓ per

min, and oxygen consumption 1.0-1.1 ℓ per min. At the moderate work rate, heart rate was 133-136 beats per min, ventilation 51-52 ℓ per min, and oxygen consumption 1.8-2.0 ℓ per min.

A third dive was conducted where divers breathed air at depth instead of 100% oxygen to determine whether breathing gas affected the ability to perform prolonged work in warm water. The results indicated that there were no differences between air and oxygen, except that breathing air resulted in heart rates that averaged 11 beats per min higher than with oxygen breathing.

These findings indicate that pretreatment with pyridostigmine will not affect the divers ability to perform light-moderate work for up to 3 h in warm water. Based on oxygen consumption, the data reveal that this type of dive could be completed if divers were to use a closed-circuit underwater breathing apparatus (UBA). The oxygen supply of the UBA should not be a limiting factor for the type of dive simulated in this study. On the other hand, based on respiratory minute ventilation, the amount of gas required if open-circuit scuba were to be used would exceed the capacity of a standard set of double tanks.

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TABLE 1: SUBJECT PHYSICAL CHARACTERISTICS

Subject Number	Age (yrs)	Height (cm)	Weight (kg)	% body fat	$\dot{V}_{E_{max}}$ (ℓ/min)	$\dot{V}_{O_{2max}}$ (ml/min/kg)	HR_{max} (bpm)
1	33	182	74.46	13	129	39	200
2	31	184	92.20	15	189	50	201
3	27	172	64.86	14	179	48	193
4	28	183	79.14	12	139	42	185
5	28	170	70.12	15	144	45	188
6	37	177	80.34	20	147	40	178
7	25	182	74.36	11	142	50	199
8	33	183	94.22	21	153	35	187
9	29	183	86.40	12	147	43	178
10	29	178	76.68	15	168	51	186
MEAN	30	179	79.28	15	154	44	190
SD	4	5	9.36	3	19	5	9

% body fat measured by bioelectric impedance

$\dot{V}_{E_{max}}$, $\dot{V}_{O_{max}}$, and HR_{max} obtained during an incremental cycle ergometer test conducted in the dry, 5-10 days prior to first test exposure. Workload began at 50 W and increased 50 W every 2 min.

TABLE 2: HEART RATE DURING REST & EXERCISE
(beats/min, mean ± SEM)

	DRUG	PLACEBO	AIR
25 W WORKLOAD	115 ± 3	112 ± 3	115 ± 4
POST 25 W REST	88 ± 2	88 ± 2	89 ± 4
1 W/kg WORKLOAD	133 ± 4 #	136 ± 4 #	145 ± 4 #*
POST 1 W/kg REST	100 ± 5 @	94 ± 4 @	106 ± 2 @

# $p < 0.001$ from corresponding 25 W
* $p < 0.02$ from corresponding placebo
@ $p < 0.001$ from corresponding 25 W REST

FIGURE LEGENDS

FIGURE 1: Minute ventilation ($\dot{V}_E$) during immersion for pyridostigmine (PYR, filled circles), placebo (PLA, open circles), and AIR (open triangles) trials. Number of subjects measured at each point varied from 3-10.

FIGURE 2: Top panel: $\dot{V}_E$ averaged at each of the exercise and rest periods for 9 subjects completing both the drug and placebo tests. Lower panel: oxygen consumption ($\dot{V}_{O_2}$) data for same subjects.

FIGURE 3: Top panel: $\dot{V}_E$ data for 8 subjects completing the AIR and O_2 (no drug) tests. Lower panel: $\dot{V}_{O_2}$ data for same subjects.

FIGURE 4: Oxygen consumption ($\dot{V}_{O_2}$) data. Symbols and abbreviations are the same as in Figure 1. Number of subjects measured at each point: 3-10.

FIGURE 5: Ventilatory equivalent ($\dot{V}_E/\dot{V}_{O_2}$) for PYR and PLA trials averaged during the 3 work periods at 25 W (0-110 min) and during the 3 periods at 1 W/kg (125-175 min).

FIGURE 6: $\dot{V}_E/\dot{V}_{O_2}$ for AIR and PLA trials. Bars have the same temporal representation as in Figure 5.

FIGURE 7: Tidal volume (top panel) and breathing frequency (lower panel) for PYR and PLA trials. Number of subjects measured at each point: 3-10.

FIGURE 8: Tidal volumes (top panel) and respiratory frequencies (lower panel) for air and 100% oxygen breathing.

FIGURE 9: Respiratory timing patterns for an pyridostigmine and placebo groups.

FIGURE 10 :Respiratory timing patterns for air and 100% oxygen breathing. (Asterix indicates $p<0.05$ between groups).

FIGURE 11: Respiratory control variables for pyridostigmine and placebo treatment groups. (Asterix indicates $p<0.05$ between groups).

FIGURE 12: Respiratory control variables for air and 100% oxygen breathing. (Asterix indicates $p<0.05$).

FIGURE 13: Heart rate for PYR, PLA, and AIR trials. Number of subjects measured at each point 7-10.

FIGURE 14: Relative perceived exertion (RPE) scores for PYR, PLA, and AIR trials. Number of subjects measured at each point: 9 for PYR and PLA, 8 for AIR.

FIGURE 1

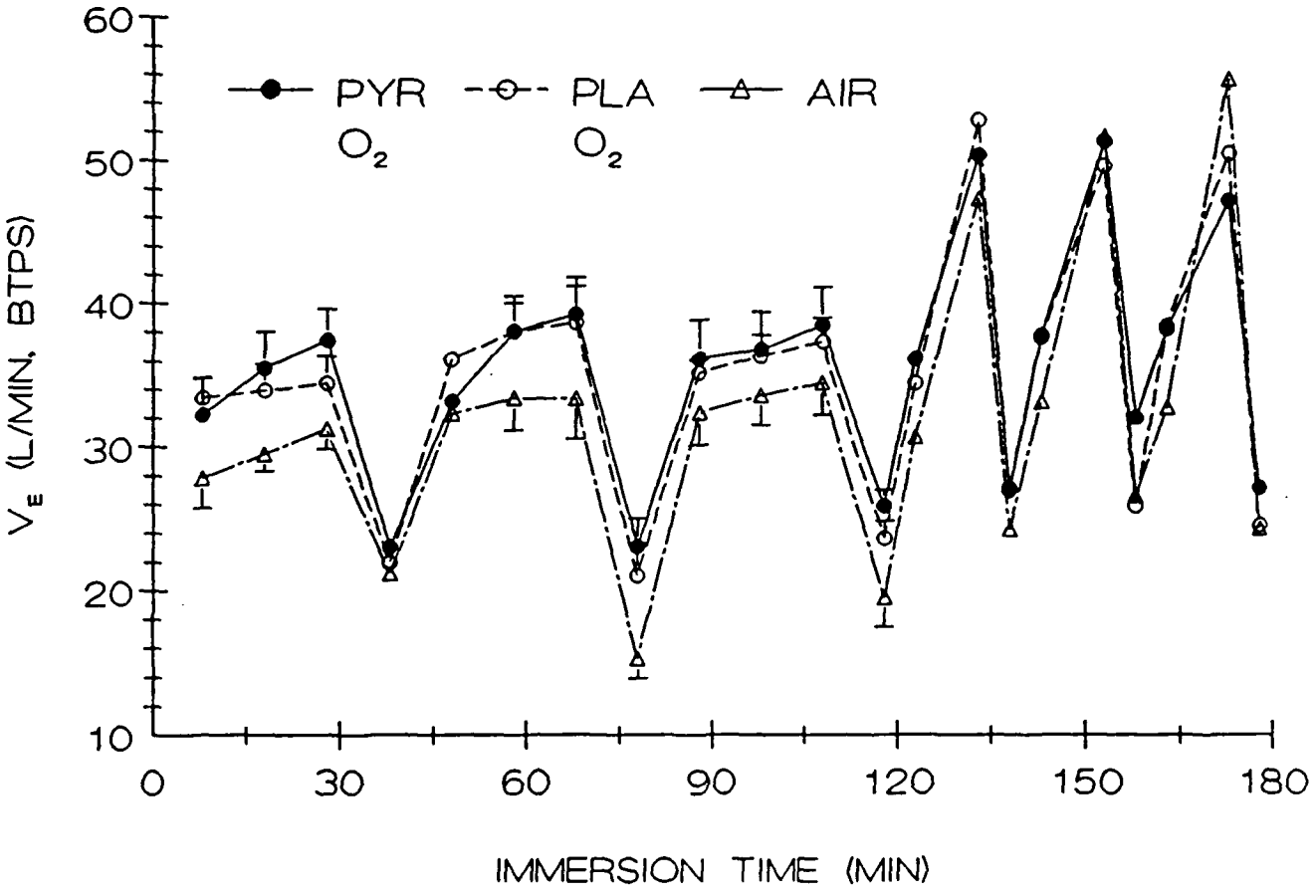

60
50
40
30
20
10
PYR O_2
PLA O_2
AIR
V_E (L/MIN, BTPS)
0
30
60
90
120
150
180
IMMERSION TIME (MIN)

FIGURE 2

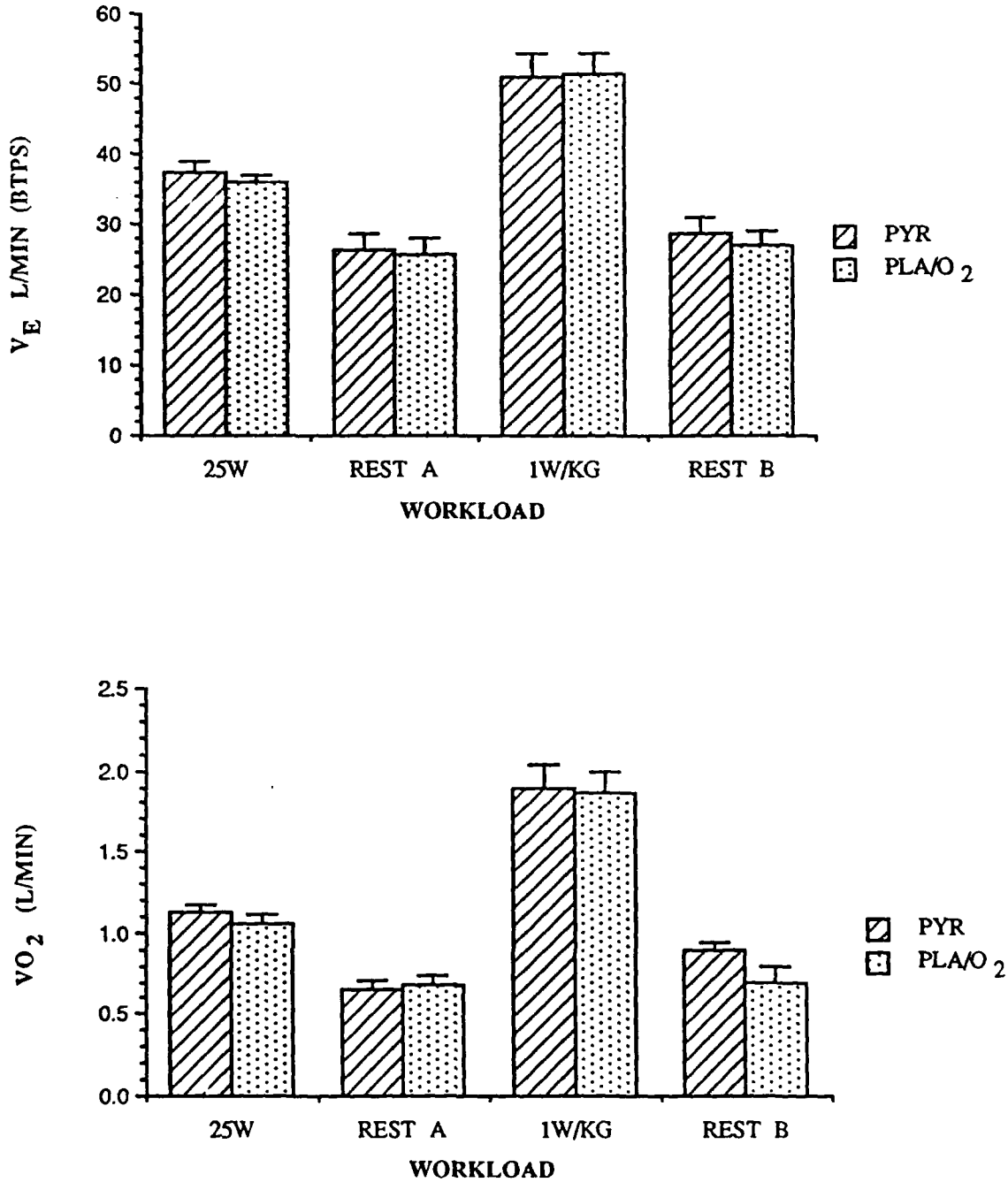
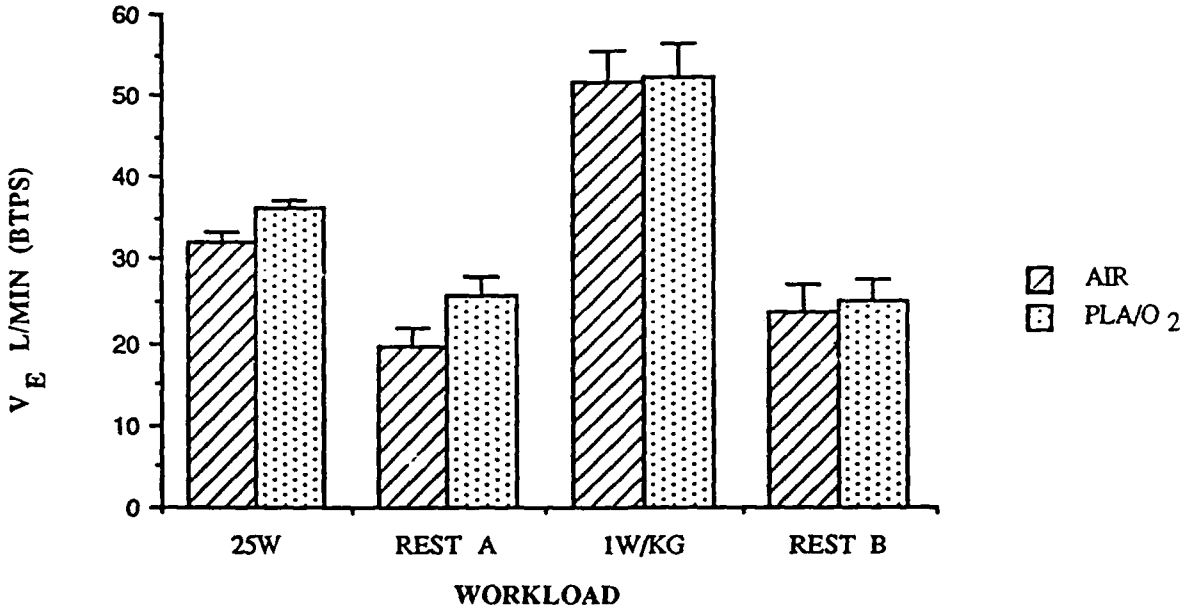

60
50
40
30
20
10
0
V_E L/MIN (BTPS)
25W
REST A
1W/KG
REST B
WORKLOAD
PYR
PLA/O_2
2.5
2.0
1.5
1.0
0.5
0.0
VO_2 (L/MIN)
25W
REST A
1W/KG
REST B
WORKLOAD
PYR
PLA/O_2

FIGURE 3


60
50
40
30
20
10
0
V_E L/MIN (BTPS)
25W
REST A
1W/KG
REST B
WORKLOAD
AIR
PLA/O_2

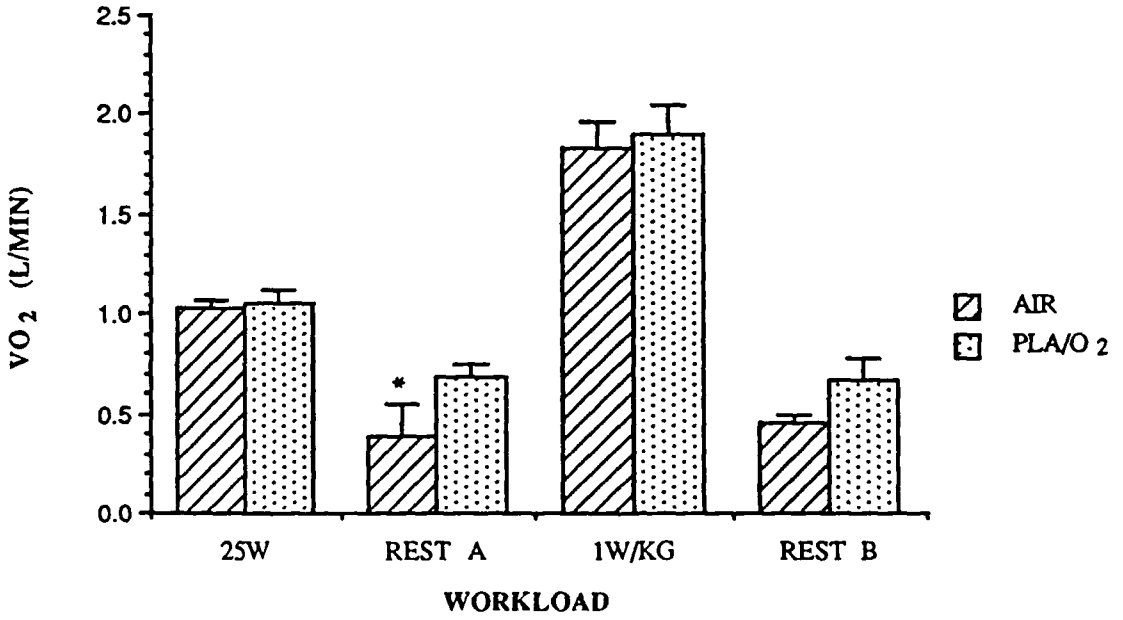

2.5
2.0
1.5
1.0
0.5
0.0
VO_2 (L/MIN)
*
25W
REST A
1W/KG
REST B
WORKLOAD
AIR
PLA/O_2

FIGURE 4

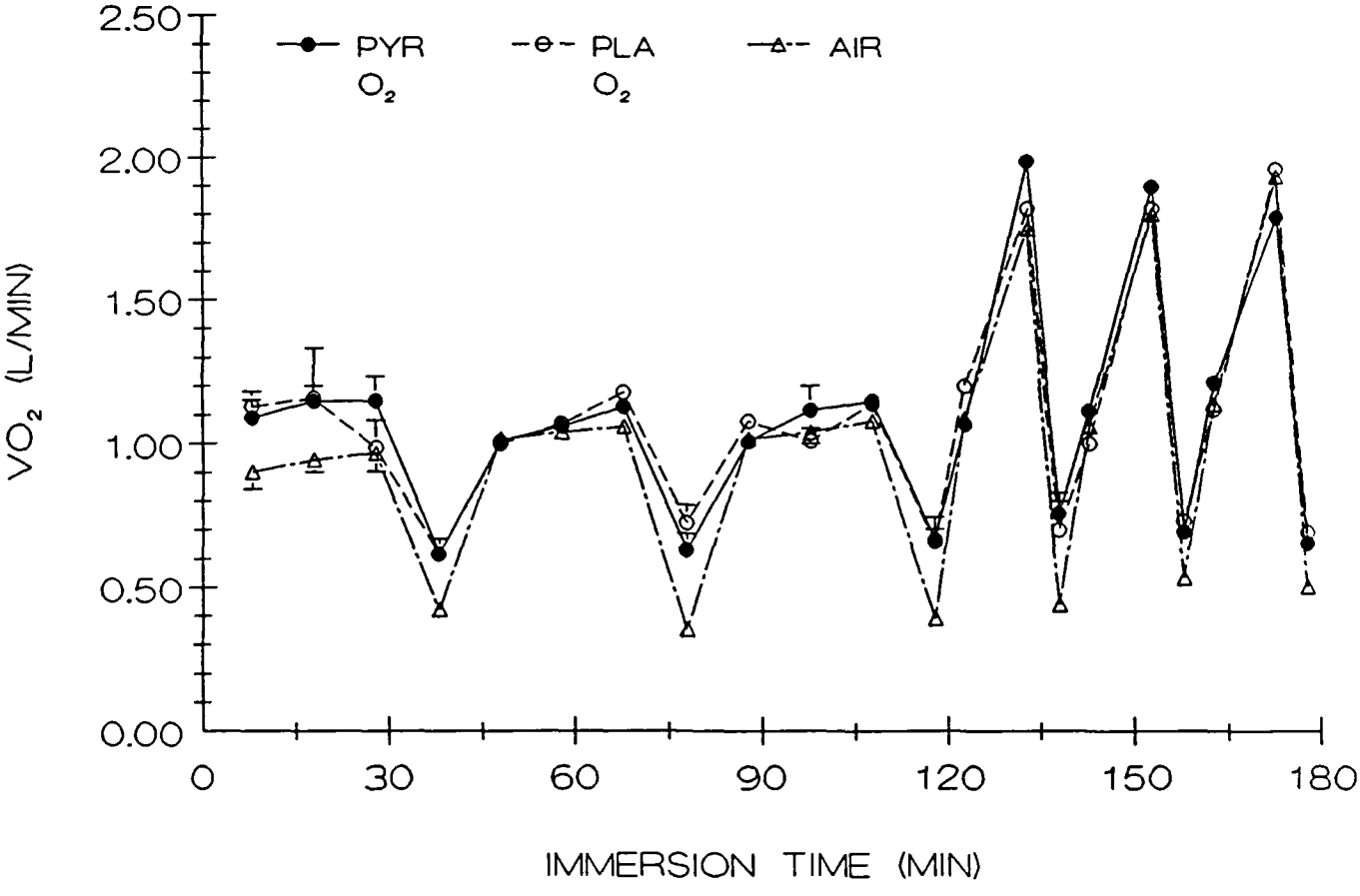

PYR
O_2
PLA
O_2
AIR
2.50
2.00
1.50
1.00
0.50
0.00
VO_2 (L/MIN)
0
30
60
90
120
150
180
IMMERSION TIME (MIN)

FIGURE 5

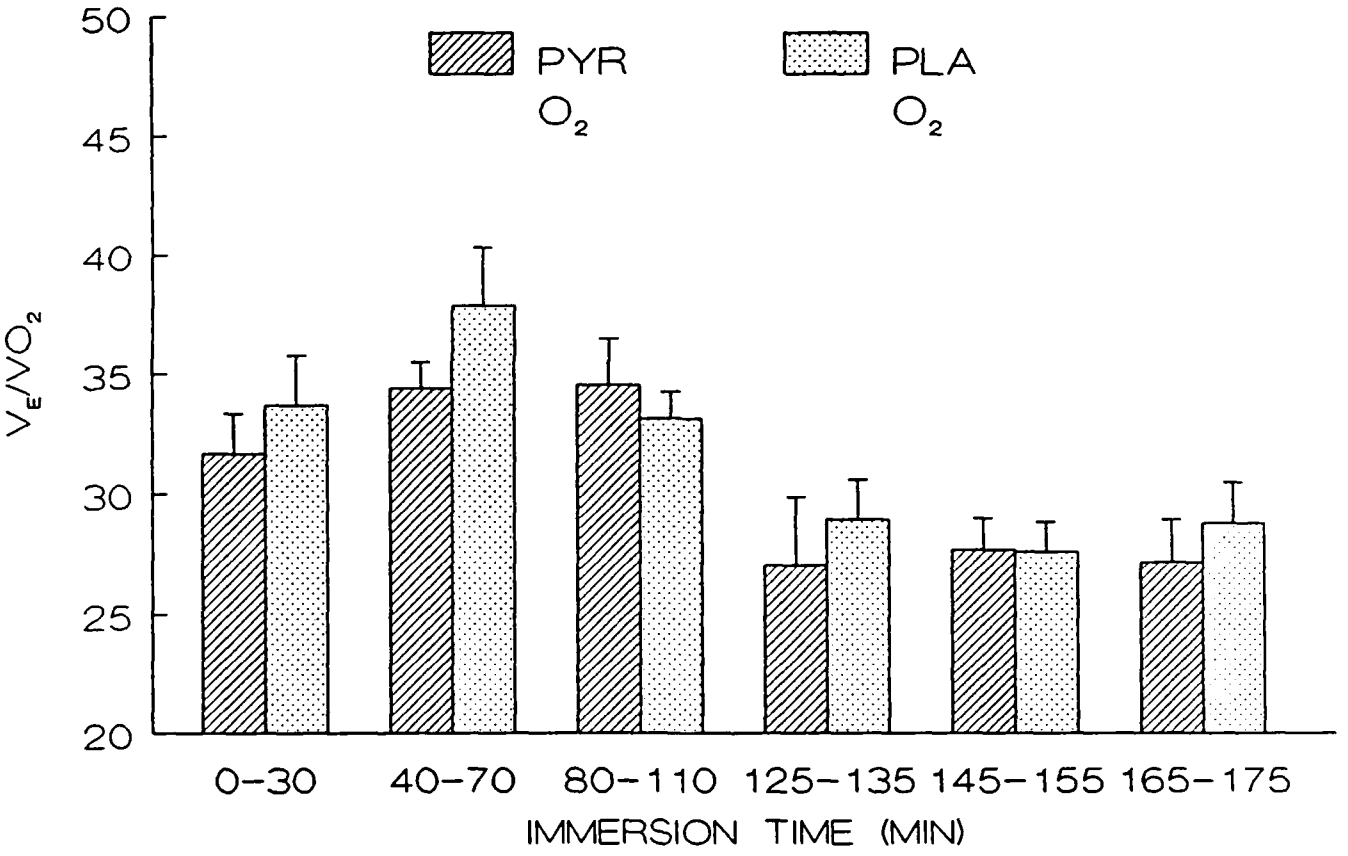

50
45
40
35
30
25
20
V_E/VO_2
PYR
O_2
PLA
O_2
0–30
40–70
80–110
125–135
145–155
165–175
IMMERSION TIME (MIN)

FIGURE 6

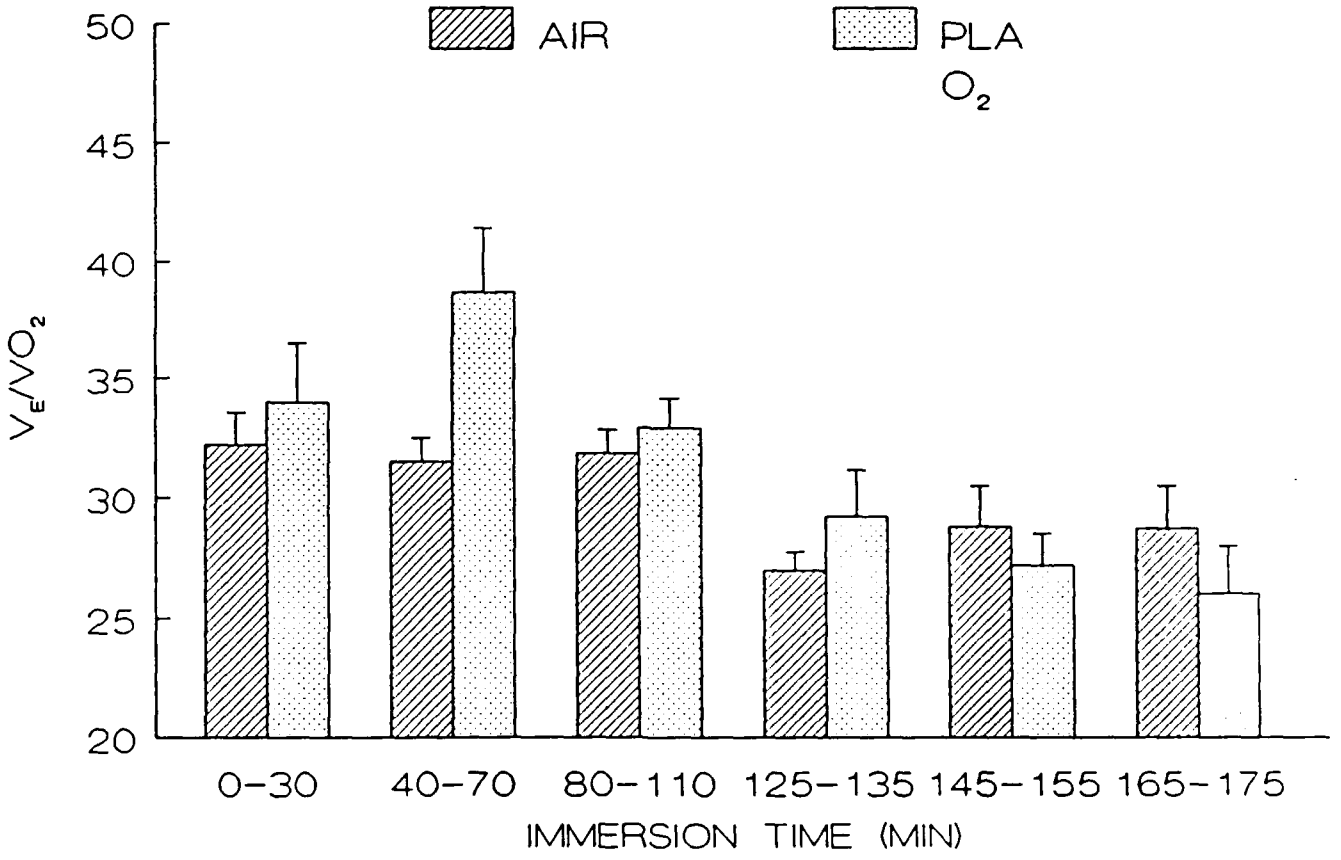

AIR
PLA
O_2
50
45
40
35
30
25
20
V_E/VO_2
0–30
40–70
80–110
125–135
145–155
165–175
IMMERSION TIME (MIN)

FIGURE 7

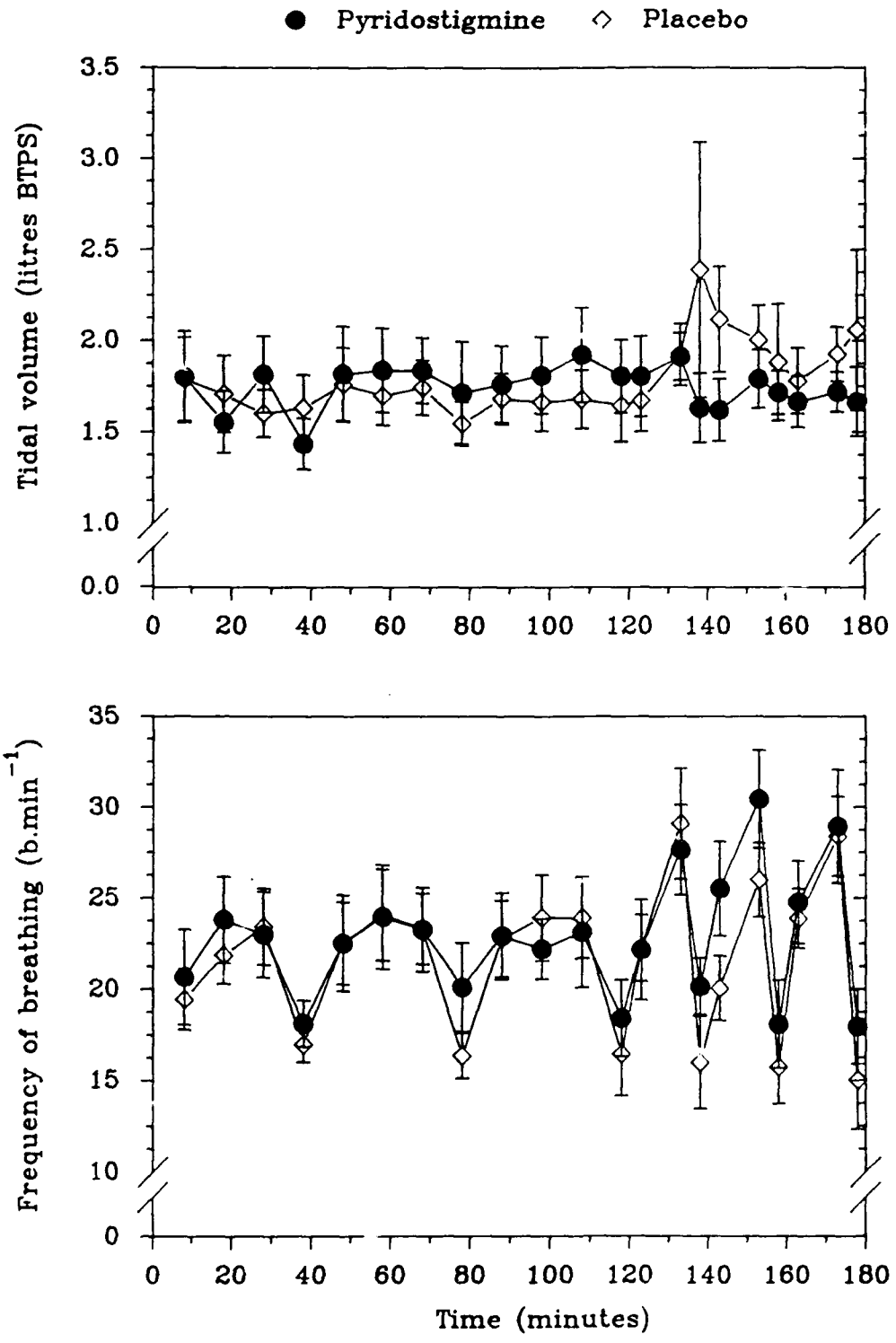

Pyridostigmine
Placebo
Tidal volume (litres BTPS)
3.5
3.0
2.5
2.0
1.5
1.0
0.0
0
20
40
60
80
100
120
140
160
180
Frequency of breathing (b.min^{-1})
35
30
25
20
15
10
0
Time (minutes)

FIGURE 8

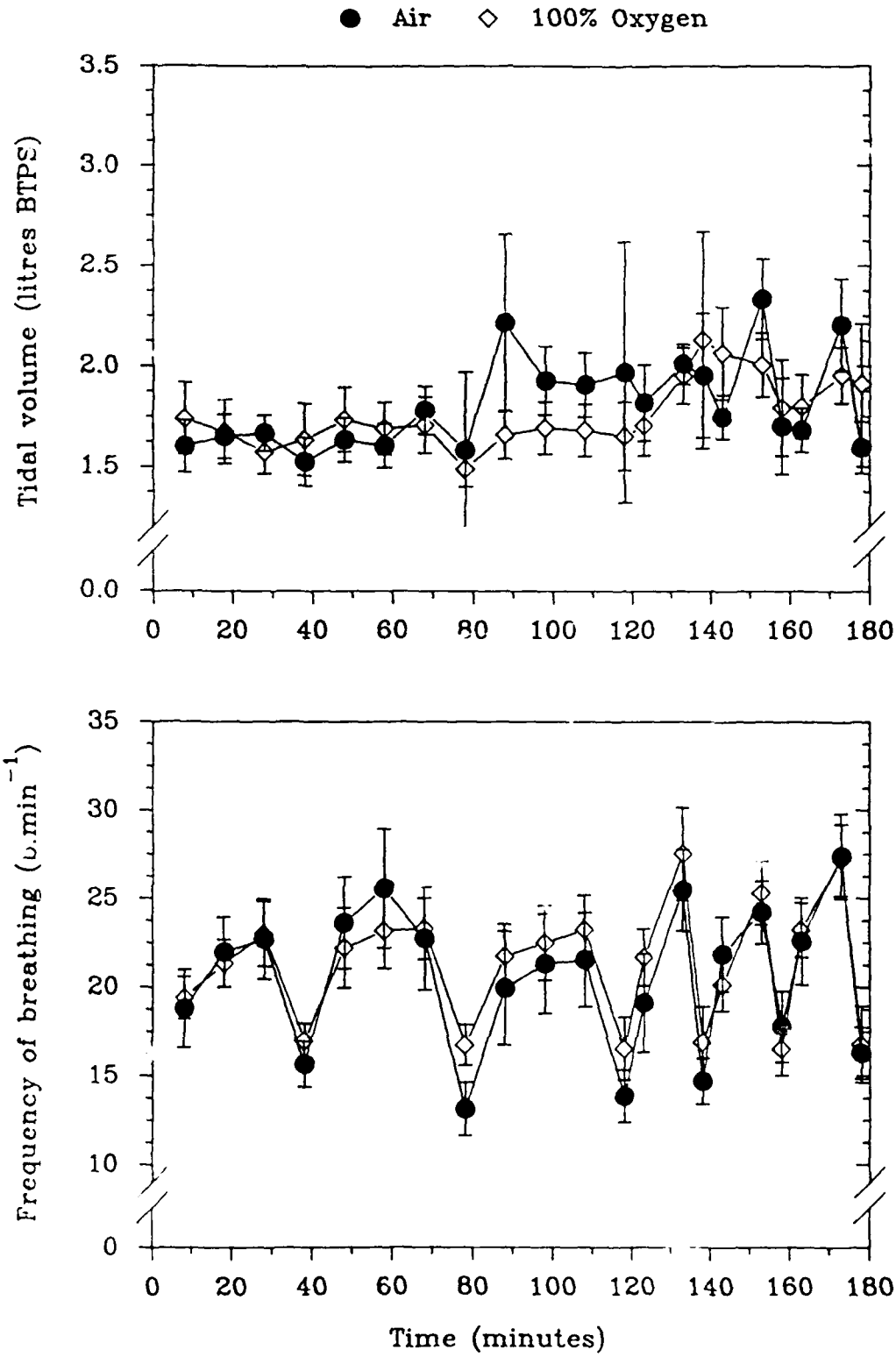

Air
100% Oxygen
Tidal volume (litres BTPS)
3.5
3.0
2.5
2.0
1.5
0.0
0
20
40
60
80
100
120
140
160
180
Frequency of breathing (ω.min⁻¹)
35
30
25
20
15
10
0
Time (minutes)

FIGURE 9

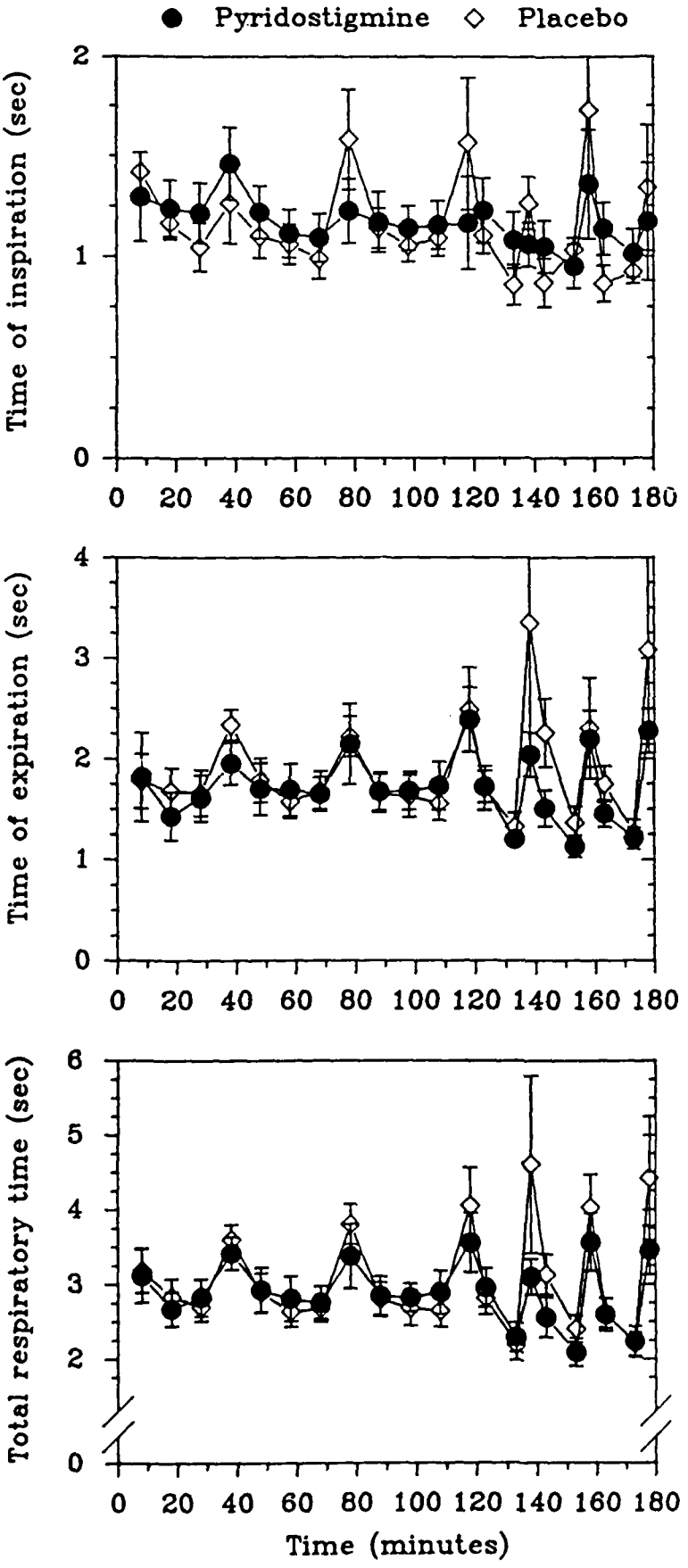

Pyridostigmine
Placebo
Time of inspiration (sec)
Time of expiration (sec)
Total respiratory time (sec)
Time (minutes)
0
20
40
60
80
100
120
140
160
180

FIGURE 10

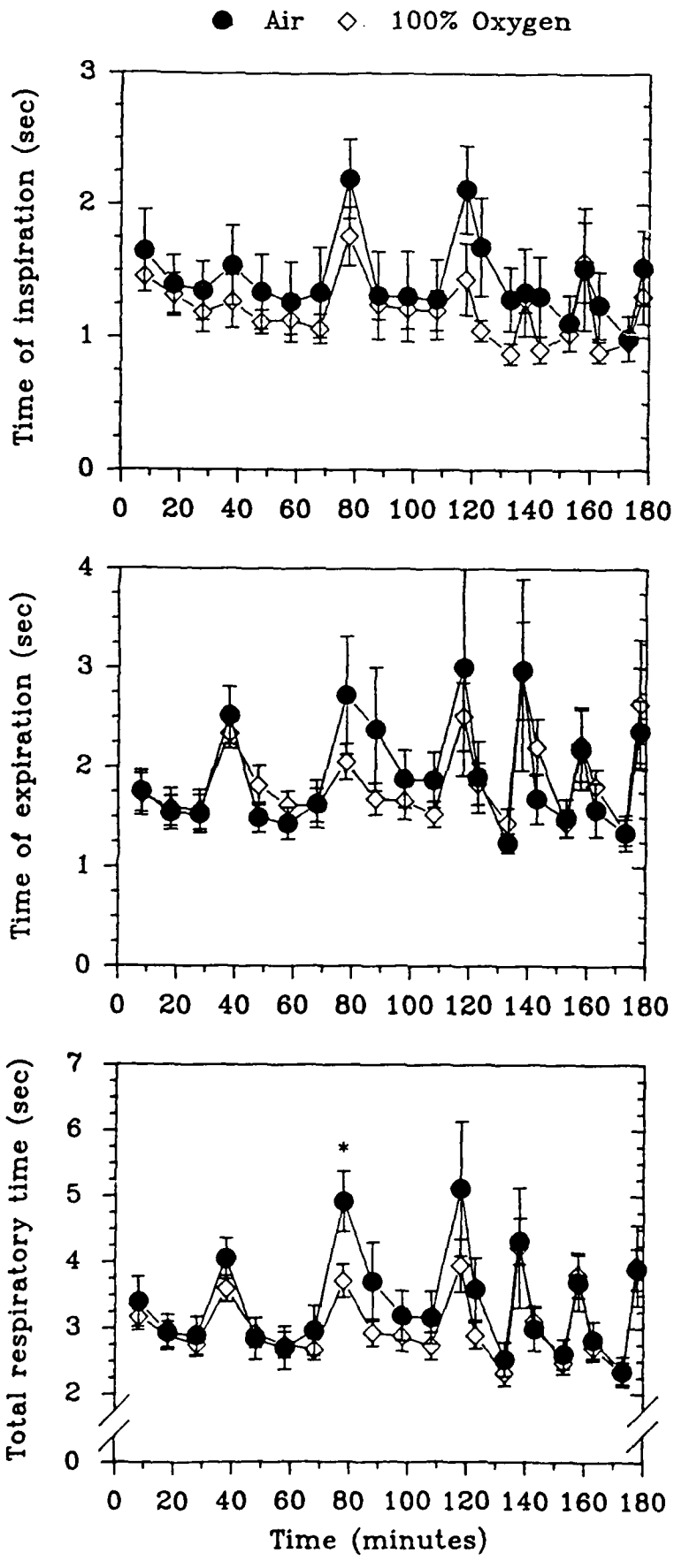

● Air ◇ 100% Oxygen
Time of inspiration (sec)
Time of expiration (sec)
Total respiratory time (sec)
*
Time (minutes)

FIGURE 11

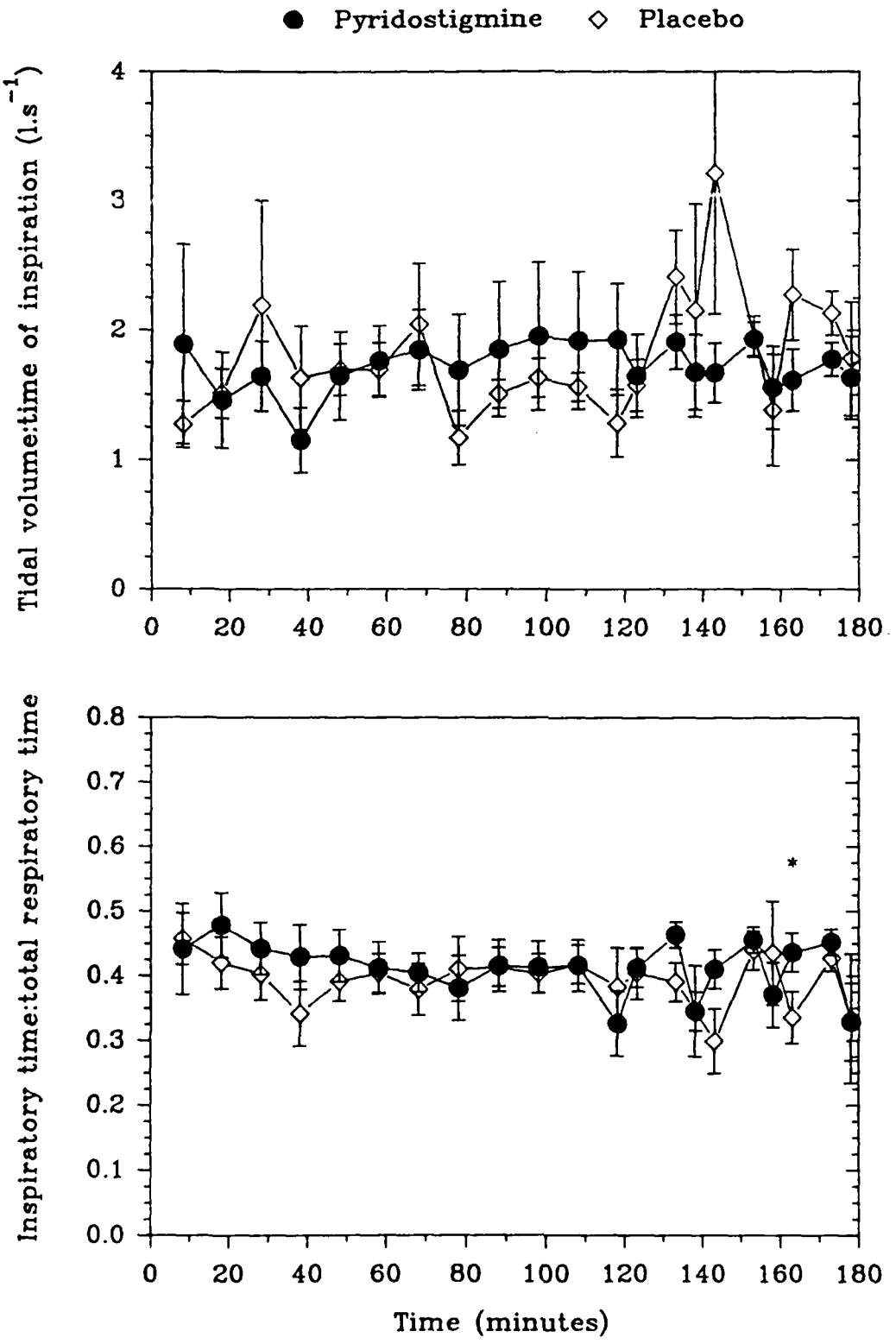

Pyridostigmine
Placebo
Tidal volume:time of inspiration (l.s^{-1})
Inspiratory time:total respiratory time
Time (minutes)
*

FIGURE 12

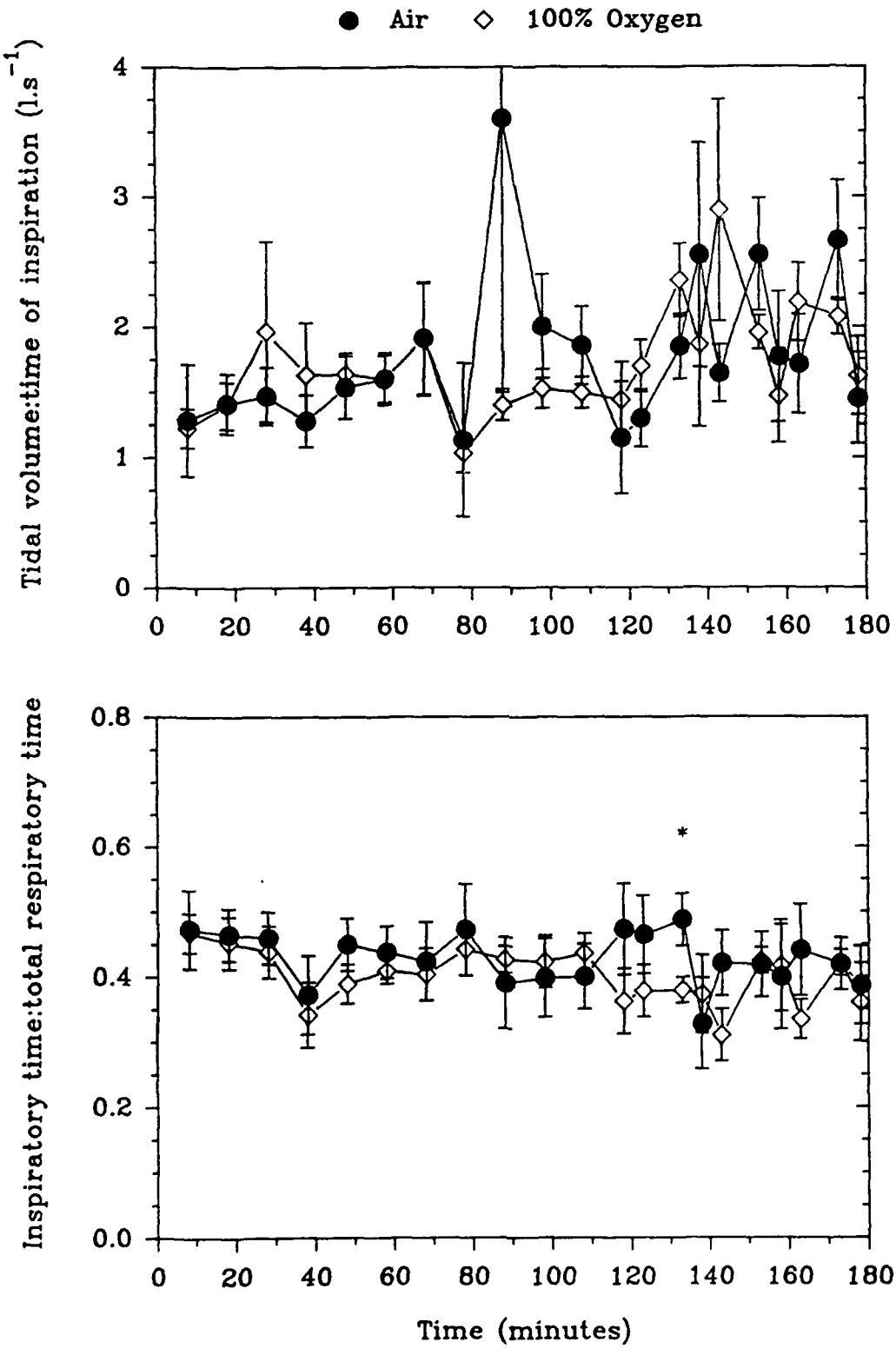

Air
100% Oxygen
Tidal volume:time of inspiration (l.s^{-1})
Inspiratory time:total respiratory time
Time (minutes)
0
1
2
3
4
0.0
0.2
0.4
0.6
0.8
0
20
40
60
80
100
120
140
160
180
*

FIGURE 13

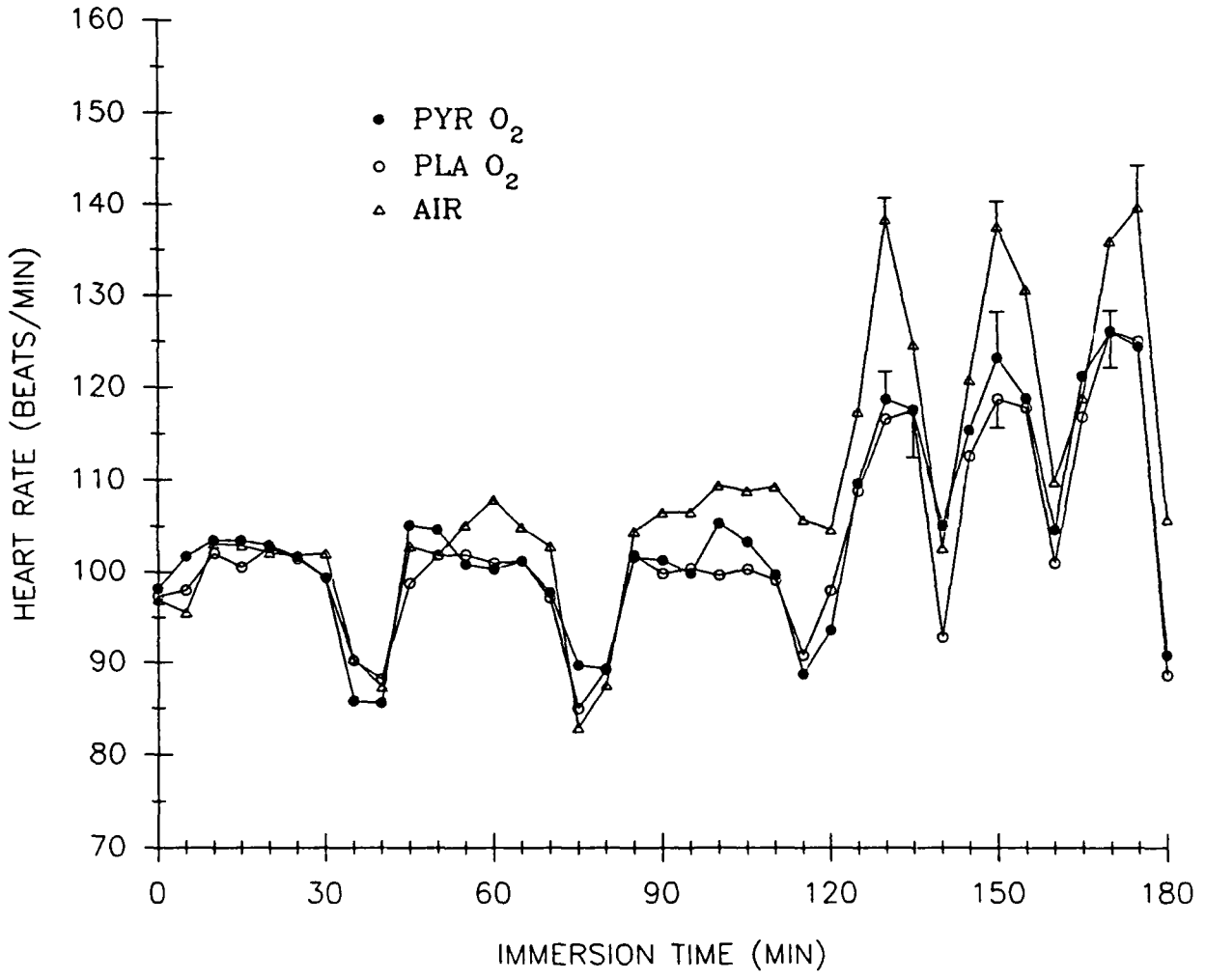
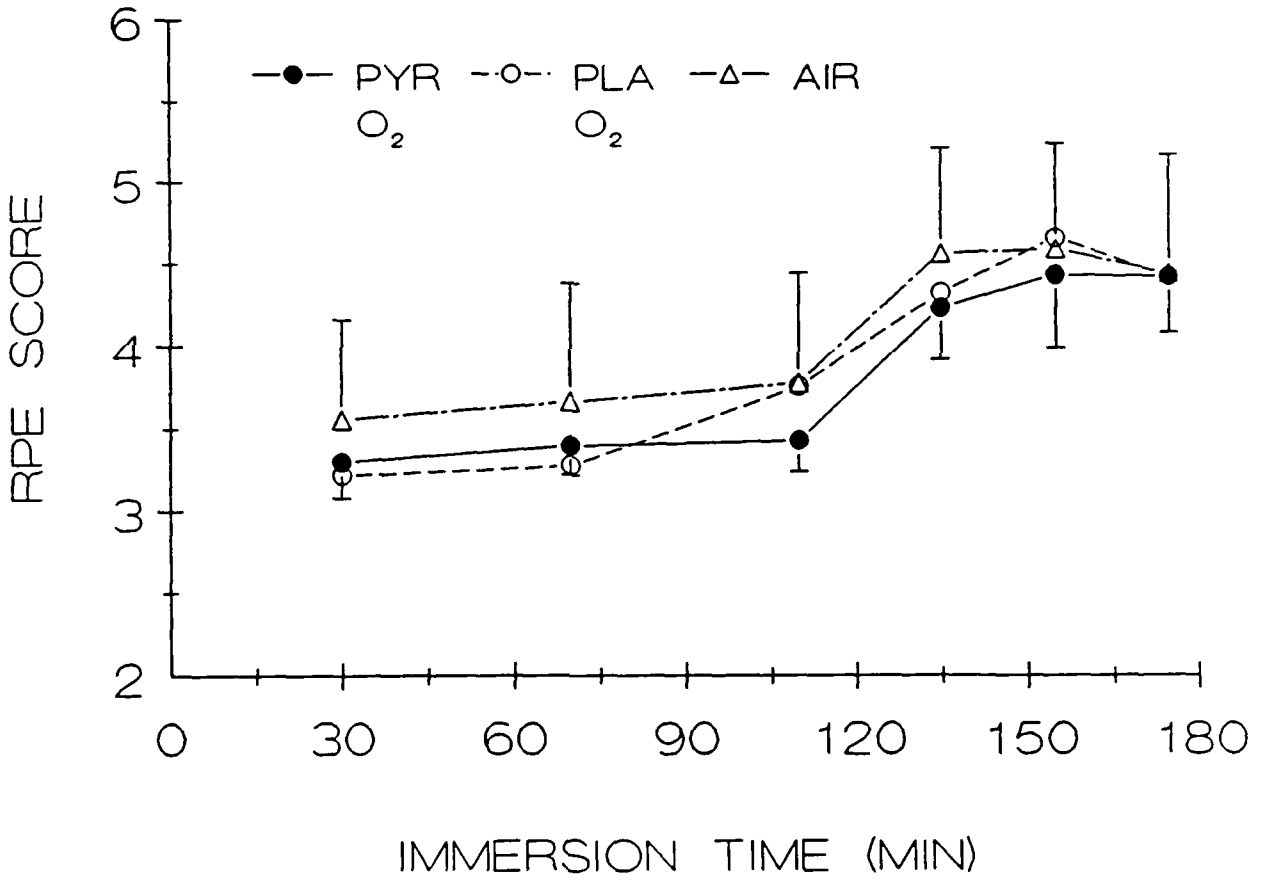

160
150
140
130
120
110
100
90
80
70
HEART RATE (BEATS/MIN)
PYR O_2
PLA O_2
AIR
0
30
60
90
120
150
180
IMMERSION TIME (MIN)

FIGURE 14


6
5
4
3
2
RPE SCORE
PYR
O_2
PLA
O_2
AIR
0
30
60
90
120
150
180
IMMERSION TIME (MIN)